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Glutathione Depletion and Insecticide Synergism in *Triatoma infestans* produced by the Butyl Ester of Buthionine Sulfoximine

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Abstract: The butyl ester of buthionine sulfoximine (BBSO) applied topically to the nymph V stage of *Triatoma infestans* (Klug) caused glutathione depletion which was maintained for four days after treatment. Topical pre-treatment of nymph V with BBSO significantly synergised the toxicity of DDT and fenitrothion to *T. infestans*.

Keywords: glutathione depletion, *Triatoma infestans*, BBSO.

1 INTRODUCTION

The tripeptide, glutathione, is found in animal, plant and bacterial cells, where it is principally related to amino acid transport through membranes and to the maintenance of redox cellular status.[1] Glutathione also plays an important role in cell defence against toxic compounds, endogenous oxidative stress and in detoxification of organic xenobiotics.[2]

Changes in glutathione content have been shown to arise in many species from the action of various compounds, including L-buthionine sulfoximine (L-BSO), a specific inhibitor of γ-glutamyl cysteine synthetase, which catalyses the first step in the glutathione synthesis (Fig. 1a). At present, L-BSO is considered to be a specific glutathione depleter.[3]

Treatment with L-BSO has been shown to be effective in depleting glutathione in insects.[4] In particular, injection of *Triatoma infestans* (Klug) with L-BSO caused a reversible depletion in the abdominal content of glutathione.[5] Administration of L-BSO by feeding to the nymph II stage caused a reduction in the total glutathione content which was maintained for three days after ingestion.[5] The synergistic effect of this compound on the insecticidal action of DDT and fenitrothion, which are detoxified by glutathione-mediated processes in *T. infestans*[6,7] has also been demonstrated.[5]

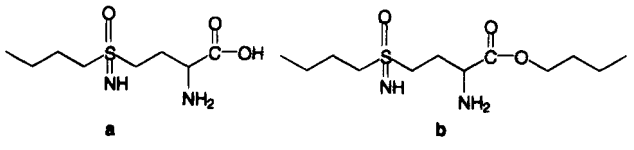

Fig. 1(a). Chemical structure of buthionine sulfoximine (BSO); (b) Chemical structure of the butyl ester of buthionine sulfoximine (BBSO).

Topical application of L-BSO is of limited effect because of the high polarity of the compound, which prevents it penetrating the insect integument. Our attempt to deplete glutathione or synergise the insecticidal activity of DDT or fenitrothion by topical application of L-BSO were unsuccessful. As a working hypothesis, we considered that esterification of the COOH group could improve the ability of the compound to reach the haemolymph of *T. infestans*, where the presence of highly active esterases has been shown.[8]

In the present study, we have synthesised the butyl ester derived from BSO (Fig. 1b), and analysed its activity in depleting glutathione and its synergistic effect on the insecticidal activity of DDT and fenitrothion against *T. infestans*. This is an extension of previous work searching for novel mechanisms of insecticide synergism relating to the control of *T. infestans*, the most important vector of Chagas disease in Argentina.[9,10]

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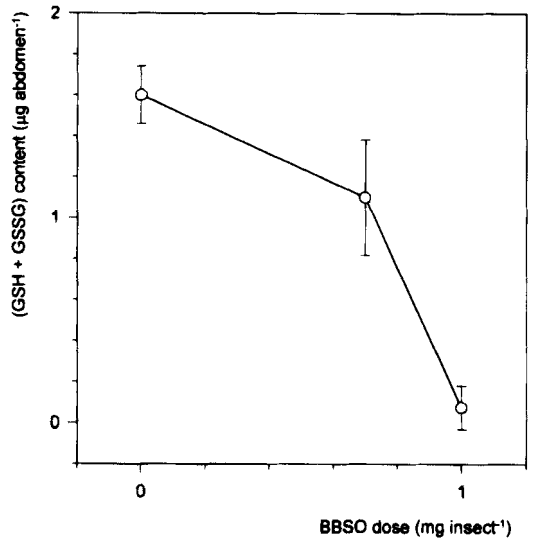

Fig. 2. Abdominal glutathione content of *Triatoma infestans* nymph V two days after topical treatment with different doses of BBSO (700 µg and 1 mg per insect). Data are the means of at least two independent determinations.

2 MATERIALS AND METHODS

2.1 Insects

Nymphs V of *T. infestans* were from our colony reared at 50–60% RH, 30°C, fed on pigeons. Mean weight = 70·0(±2·3) mg.

2.2 Chemicals

DDT, 99% Pesticide Standard, from Dualabos (USA). Fenitrothion, 96% was a gift from Sumitomo Chemical Co., Ltd (Japan). Other chemicals were obtained from Sigma or Aldrich (USA).

2.3 Synthesis of butyl ester of buthionine sulfoximine (BBSO)

Racemic BSO was treated with butanol and thionyl chloride as acid catalyser.[11] After neutralisation of the reaction product with ammonium hydroxide solution, BBSO was extracted with chloroform and purified on a silica gel column using chloroform as eluent. The solvent was removed by rotary evaporation. HPTLC analysis using methanol + water (80 + 20 by volume) showed only one spot ($Rf = 0{\cdot}6$). The oily compound was characterised by [^{1}H] NMR (deuterochloroform): δ 1·0 (t, 3H), 1·1 (t, 3H), 1·4 (m, 8H), 1·7 (m, 2H), 2·9 (s, 1H), 3·4 (t, 4H), 3·8 (s, 2H), 3·8 (t, 2H), 3·9 (t, 1H) ppm.

2.4 BBSO administration and glutathione + GSSG determination

Nymph V were topically treated on the dorsal abdomen with 7 or 10 µl of an acetone solution of BBSO (100 mg ml^{-1}); control insects were treated with the same volume of acetone. Two or four days after treatment, insects were dissected to reveal the abdominal contents, from which the gut was removed and exhaustively washed. The abdominal internal systems without gut from three insects were homogenised in distilled water (1 ml), and protein was precipitated with trichloroacetic acid (25 g litre^{-1}). After centrifuging for 10 min at 5000*g*, the supernatant was adjusted to pH 7 with potassium hydroxide, and glutathione and GSSG determined enzymatically by the method of Owens and Belcher.[12]

2.5 Synergism bioassays

Groups of seven-day-old nymphs ($n = 10$), starved from moulting, were topically treated on the dorsal abdomen with acetone (10 µl) or an acetone solution of BBSO (100 mg ml^{-1}; 10 µl). Two days later, acetone (5 µl) or an acetone solution of DDT (60 mg ml^{-1}, 5 µl) or fenitrothion (0·1 mg ml^{-1}; 6 µl) was topically applied to the ventral abdomen. Mortality was determined after one or two days. Assays were carried out in duplicate. At the dose assayed BBSO applied alone in acetone has no insecticidal effect.

3 RESULTS AND DISCUSSION

The aim of this study was to find a derivative compound that would improve the integumental penetration of L-buthionine sulfoximine (L-BSO), while conserving its action as an abdominal glutathione depletor in *T. infestans*, the principal vector of Chagas disease in Latin America.[5] Such a depletion has a synergistic effect on the insecticidal action of DDT and fenitrothion.[5]

The first stage of the movement of a compound into an insect appears to be a simple dissolution into the epicuticular wax.[13] The high polarity of L-BSO makes this difficult. In such cases, derivatisation of the polar groups has been shown to improve toxicological properties.[14] In the present study, the butyl ester derived from BSO (BBSO) was synthesised for this purpose. It was assumed that, after penetration through the integument, BBSO would be hydrolysed by the haemolymph esterase previously described in *T. infestans*.[8,15]

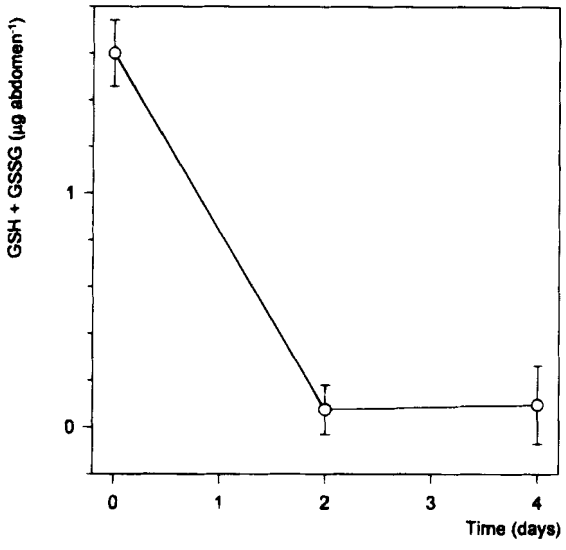

Fig. 3. Time course of the glutathione depletion in abdominal internal systems without gut of nymphs V of *Triatoma infestans* after topical application of BBSO (1 mg per insect). Data are the means of at least two independent determinations.

The glutathione content of the abdominal internal systems, without gut, of nymph V of *T. infestans* was measured 48 h after topical application of BBSO to the dorsal abdomen. At both doses assayed (700 μg and 1 mg per insect), BBSO caused a decrease in the glutathione content, reducing this to undetectable levels at the higher dose (Fig. 2). At this higher dose, the effect was detectable up to four days after application (Fig. 3).

The effect of BBSO on the insecticidal activity of DDT against *T. infestans* is shown in Fig. 4. BBSO

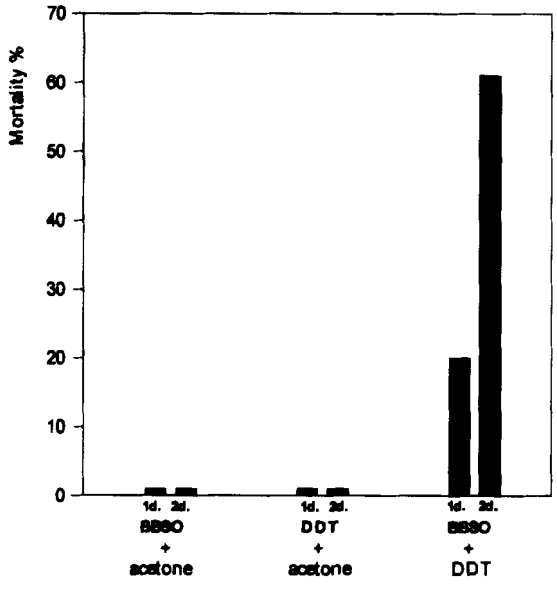

Fig. 4. Effect of BBSO pretreatment on the mortality of nymphs V of *Triatoma infestans* produced by topical application of DDT two days later. BBSO: 1 mg per insect topically applied on dorsal abdomen. DDT: 300 μg per insect topically applied on ventral abdomen.

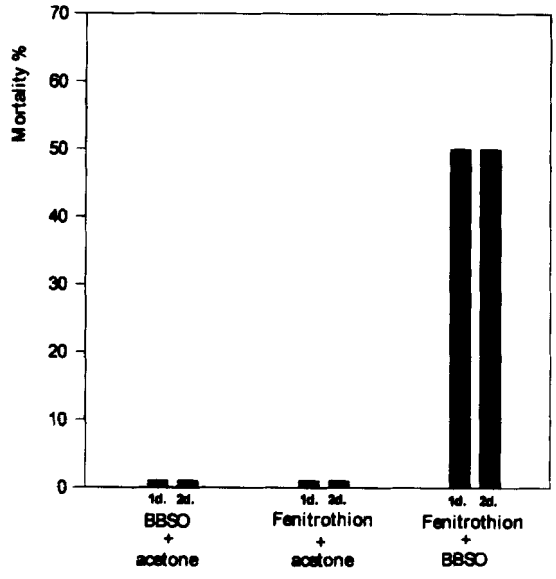

Fig. 5. Effect of BBSO pretreatment on the mortality of nymphs V of *Triatoma infestans* produced by topical application of fenitrothion two days later. BBSO: 1 mg per insect topically applied on dorsal abdomen. Fenitrothion: 0·6 μg per insect topically applied on ventral abdomen.

applied two days before the application of a sub-lethal dose of DDT produced an increase in mortality mainly evident two days after DDT application. The low activity of DDT against *T. infestans* has previously been analysed on the basis of two toxicokinetic steps: a very slow penetration into starved insects[16] and a very efficient detoxification metabolism mediated by glutathione.[6] If the latter step is blocked by BBSO, an insecticidal affect will be apparent after the slow DDT penetration. In this respect, the sustained depleting activity of BBSO over at least four days improves its ability to potentiate DDT action.

The effect of topical pretreatment with BBSO on the insecticidal activity of fenitrothion against nymph V of *T. infestans* is shown in Fig. 5. At the dose applied (0·6 μg per insect), high mortality was only achieved after pretreatment with BBSO. This is in line with previous work in our laboratory which showed fenitrothion degradation in *T. infestans* mediated by glutathione[7] and synergism of the insecticide by either injection or feeding with a solution of L-BSO.[5]

4 CONCLUSIONS

The results described above show that the synergism of BBSO on the insecticidal action of DDT and fenitrothion in *T. infestans* is related to glutathione depletion. Taken together with our previous results on L-BSO synergism,[5] this indicates the importance of glutathione in the degradative pathways of insecticides, and suggests

its depletion to be a possible new mechanism for the synergism of insecticides against *T. infestans*.

It is suggested that BBSO acts as a proto-synergist through its metabolic hydrolysis to BSO within the insect, but further biochemical studies are necessary to confirm this hypothesis.

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